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ESCUELA DE CIENCIAS BIOLÓGICAS

Capacidad de aclimatación en renacuajos de dos especies de anuros: *Rhinella marina* (Bufonidae) y *Gastrotheca riobambae* (Hemiphractidae) y su vulnerabilidad al cambio climático

Disertación previa a la obtención del título de Licenciada en Ciencias Biológicas

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A mis padres, María Luisa y Oswaldo,
y a mis hermanos,
Jorgito, Santy y Kari.

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LISTA DE ABREVIATURAS

ARR: Acclimation Response Ratio

ARR_{MAX}: Acclimation Response Ratio for the upper thermal limit

ARR_{MIN}: Acclimation Response Ratio for the lower thermal limit

CT: Critical Temperature/ Thermal

CTmax: Critical Temperature maximum

CTmin: Critical Temperature mínimum

Df: Degrees of freedom

L:D: Lights and Dark

MS-222: Tricaine methanesulfonate (TMS)

SD: Standard Deviation

T_{br}: Thermal breadth

Te: Exposure temperature

Te: Exposure temperature

T_{opt}: Thermal Optimum

TPC: Thermal Performance Curve

WT: Warming Tolerance

Δ: Variation

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1. RESUMEN

Cuando la temperatura ambiental cambia en un ecosistema, los organismos tienen distintas opciones para sobrellevar la modificación. La capacidad de aclimatación es una de ellas. El cambio climático actual puede estar causando declinaciones en las poblaciones de anfibios y el estudio de las tolerancias térmicas de este grupo puede permitir mejorar estrategias de conservación para las especies que se pueden ver más afectadas. En el presente estudio, dos especies de anuros, *Rhinella marina* y *Gastrotheca riobambae,* fueron expuestas a distintos tratamientos de aclimatación (14 ºC, 20 ºC, 26 ºC, 32 ºC y 36 ºC) y se obtuvo sus tolerancias térmicas para analizar si estas especies poseen capacidad de aclimatación, calculada mediante el Radio de Respuesta de Aclimatación (ARR), y evaluar su vulnerabilidad relativa al cambio climático. Los resultados mostraron una respuesta de aclimatación positiva para ambas especies, *R. marina* tuvo un ARR_{MAX}=0.2 que indica que tiene más capacidad de aclimatación hacia el calor que *G. riobambae* con ARR_{MAX}=0.12. Para el ARR_{MIN}, el resultado fue mayor aclimatación para el frío de *G. riobambae*. Esta especie mostró también tener el rango de tolerancia más amplio con 40 grados de amplitud térmica, y menor vulnerabilidad relativa al cambio climático con WT=23.6 grados.

Palabras clave: Tolerancia térmica, aclimatación, anuros tropicales, vulnerabilidad relativa al cambio climático.

2. ABSTRACT

When environmental temperature shifts in an ecosystem, organisms have different options to cope with this modification, acclimation capacity is one of them. The present climate change could be causing declines in amphibian populations and the study of thermal tolerances in this group could allow improvements in conservation strategies for species that could be easily affected. In this study, two species of anurans, *Rhinella marina* and *Gastrotheca riobambae,* were exposed to different acclimation treatments (14 ºC, 20 ºC, 26 ºC, 32 ºC and 36 ºC) in order to obtain their thermal tolerances to analyze if these species possess acclimation capacity, measured by ARR, to estimate their relative vulnerability to climate change. The results showed a positive acclimation response for both species, *R. marina* had an ARR_{MAX}=0.2 which indicates that it has more acclimation capacity to heat than *G. riobambae* with ARR_{MAX}=0.12. For the ARR_{MIN}, the result was more acclimation for cold of *G. riobambae.* This species also showed the wider thermal tolerance range with 40 degrees of thermal breadth and less relative vulnerability to climate change with WT=23.6 degrees.

Keywords: Thermal tolerance, acclimation, tropical anurans, relative vulnerability to climate change.

3. MANUSCRITO PARA PUBLICACIÓN

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Acclimation capacity in tadpoles of two anuran species: *Rhinella marina* (Bufonidae) and *Gastrotheca riobambae* (Hemiphractidae) and their vulnerability to climate change.

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Abstract

When environmental temperature shifts in an ecosystem, organisms have different options to cope with this modification, acclimation capacity is one of them. The present climate change could be causing declines in amphibian populations and the study of thermal tolerances in this group could allow improvements in conservation strategies for species that could be easily affected. In this study, two species of anurans, *Rhinella marina* and *Gastrotheca riobambae,* were exposed to different acclimation treatments (14 ºC, 20 ºC, 26 ºC, 32 ºC and 36 ºC) in order to obtain their thermal tolerances to analyze if these species possess acclimation capacity, measured by ARR, to estimate their relative vulnerability to climate change. The results showed a positive acclimation response for both species, *R. marina* had an ARR_{MAX}=0.2 which indicates that it has more acclimation capacity to heat than *G. riobambae* with ARR_{MAX}=0.12. For the ARR_{MIN}, the result was more acclimation for cold of *G. riobambae.* This species also showed the wider thermal tolerance range with 40 degrees of thermal breadth and less relative vulnerability to climate change with WT=23.6 degrees.

Keywords: Thermal tolerance, acclimation, tropical anurans, relative vulnerability to climate change.

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3.1. Introduction

Temperature is the fundamental abiotic component of the ecosystems that influences the growth, physiology, distribution patterns and organism's abundance (Hutchison, 1961; Koo et al., 2013; Tejedo et al., 2012).

In the present, our planet is suffering from climate change. There is a rise of the global temperature approximately of 0.85 (0.65 to 1.05) ºC, over the period of 1880-2012, due to higher concentrations of greenhouse gases in the atmosphere, mainly because of anthropogenic activities according to the Intergovernmental Panel of Climate Change. Trends and projections indicate that temperature is going to continue raising, more on land than in oceans (Intergovernmental Panel on Climate Change, 2014, 2007; Tejedo et al., 2012).

The effects of climate change that our planet is going through affect the ecosystems and global biodiversity at all levels, from organisms to biomes (Parmesan, 2006; Storlie et al., 2014). According to some studies, (Bellard et al., 2012; Intergovernmental Panel on Climate Change, 2014; Pacifici et al., 2015) climate change modifies ecological interactions between species of the populations inside a community, for example, between flowers and their pollinators, competition relationships, predator/prey, host/parasite or mutualism. In response to climate change, organisms have basically three options: move to new areas with the proper conditions for the species, adapt to the new changing conditions, or in the worst-case scenario, to become extinct (Bellard et al., 2012; Gerick et al., 2014).

There are different ways in which organisms can cope with changes in the environmental temperature, in a certain way, they "adjust" to change. Basically, there exist three ways animals can display responses: acute, chronic or evolutionary responses (Hill et al., 2012). One of these ways is a genetically predisposed response in a population, in which a mutation that favors adaptation and allows survival becomes evident, the evolutionary response (Salamin et al., 2010). The acute response is a rapid or immediate response, exhibit after minutes or hours after an environmental change. The chronic response is a prolonged response to new environmental conditions, such as acclimation (Charmantier et al., 2008; Hill et al., 2012). Other organisms manifest a behavioral response to

environmental changes, for example moving from sunlight to shaded places to reduce heat (Bellard et al., 2012).

One of the groups of organisms that have a high vulnerability to climate change is the ectotherms or poikilotherms, due to the fact that their basic physiological functions such as movement, growth and reproduction are strongly influenced by the environmental temperature (Deutsch et al., 2008; Gerick et al., 2014). These organisms, with abundant diversity throughout the planet, depend on environmental temperature to regulate their own corporal temperature, they lack physiological mechanisms which allows them to thermoregulate (Hill et al., 2012). Due to global warming, studies suggest that equatorial ectotherms could be living at temperatures close to or above their physiological thermal limits, because they occupy thermal environments less variable than environments temperate species endure (Duarte et al., 2012; Gerick et al., 2014; Gutiérrez-Pesquera et al., 2016).

As a part of the response that ectotherms show towards an environmental change in their habitat, there is the acclimatization. It is a physiological chronic response by which the organism adapts to changes in the environment, expressed after prolonged exposure of few days or weeks to new environmental conditions (Angilletta, 2009; Hill et al., 2012). The only difference between acclimatization and acclimation is that the second is a laboratory phenomenon, being the response to a new environment that differs from the preceding environment in just a few, defined ways (Hill et al., 2012; Westmoreland, 1994). Acclimation is a type of phenotypic plasticity, defined as the ability of one organism (single genotype) to express two or more genetically controlled genotypes or adaptations, giving the organism the capacity of adapt to changes in their environment (Hill et al., 2012; Huey et al., 2012). There are two types of thermal acclimation: *developmental acclimation* and reversible acclimation. The first consists in irreversible responses to temperature occurred during any ontogeny stage that may enhance performance during a subsequent stage or parents could respond to temperatures during their life cycle and enhance performance of their offspring (intergenerational response) (Angilletta, 2009).

The ability of ectotherms to perform different activities according to its body temperature can be estimated through a thermal performance curve (TPC), which demonstrates the way temperature could affect main physiological functions (such as locomotion, metabolic rate,

fecundity, survival). This starts with the Critical Thermal Minimum (CTmin), then it gradually rises until the Thermal Optimum (T_{opt}) and then it drops rapidly until the Critical Thermal Maximum (CTmax) (Gerick et al., 2014; Huey and Stevenson, 1979). The concept of CTmax was introduced by Cowles and Bogert (1944) and is defined as the "thermal point at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death". CTmax and CTmin are the highest and lowest temperature at which the organism can react to external stimuli (Hutchison, 1961). Those are also the limits of the thermal tolerance range or thermal performance breadth (T_{br}), being that outside those limits, it would imply the death of the organisms, and each thermal tolerance range is different to each species (Gerick et al., 2014; Tejedo et al., 2012).

Amphibians are the vertebrate ectotherms that have presented the most drastic population decline in recent years, according to UICN (Hoffmann et al., 2010). Amphibian skin serves them as an osmorregulatory and respiratory organ, for these and many other reasons, they are highly sensitive to minimal environmental changes (Bernal and Lynch, 2013; Hill et al., 2012; Tejedo et al., 2012). The decline of amphibian populations in the last four decades, is not only due to climate change, scientists have to consider complex interactions of several anthropogenic factors, for example, fragmentation and loss of habitat, pollution, diseases (Collins and Storfer, 2003; Pounds et al., 2006).

Given its high sensitivity to temperature or humidity changes in their environments, their unique skin qualities and the fact of being an ectotherm, amphibians are great bioindicators of their environment, and studying amphibian declines may serve to understand other species declines too (Angulo, 2002; Collins and Storfer, 2003; Duellman, 2015; Viernum, 2012). The larval phase of amphibians, tadpoles, are part of a lifecycle that some amphibians go through, with several other reproductive strategies (Duellman, 2015). Amphibians have nearly 40 reproductive strategies, some even with direct development from egg to juvenile (e.g. the genus *Pristimantis*) but the aquatic reproductive strategy is considered the ancestral way (Duellman and Trueb, 1986; Woodley, 2015). Commonly, tadpoles inhabit water bodies as temporary ponds, in which they could be exposed to physiological stress, which probably indicates a selective pressure for the individuals to bear a wide variation of the water temperature, depending on the hour of the day, the sun's

position, or the location of the pond itself (Gutiérrez-Pesquera et al., 2016; Tejedo et al., 2012).

The study of the thermal limits of specific species is important: it allows for determining the vulnerability of the specie (Charmantier et al., 2008). The vulnerability of determined species is studied by obtaining the physiological data, and comparing them to the temperature of its environment (microhabitat) at which the organisms are normally exposed, to check if the specie is normally exposed to its physiological limits inside it's natural habitat, and check for possible impacts that climate change could have over the specie (Gutiérrez-Pesquera et al., 2016; Storlie et al., 2014; Tejedo et al., 2012).

The study subjects, *Rhinella marina* and *Gastrotheca riobambae,* belong to the anuran families Bufonidae and Hemiphractidae respectively. *Gastrotheca riobambae* is found near water sources such as irrigation channels, water shafts, creeks, small swamps or marshes, including man intervened areas. The special feature about this species is a characteristic of the family, the marsupial frogs. When they reproduce, the female carries the eggs in a pouch on her back until the tadpoles hatch and are released into water bodies, in the case of some species, or directly as juveniles in others (Duellman, 2015). The distribution range of this endemic species is restricted to the Ecuadorian Highland, between the 1578 and 3500 m.a.s.l. and it is distributed throughout forests and interandean valleys of the north and center of the country (Frenkel et al., 2014).

The other species, *Rhinella marina*, has a wide distribution range in 9 out of the 10 natural regions of Ecuador, only absent in the Paramo (Coloma et al., 2014). It is a native species of America, present both north and south of the continent, and also has been introduced to different parts of the world like Australia, where it is consider a plague (Jolly et al., 2015; McCann et al., 2014; Tingley and Shine, 2011). Considering these facts, this specie has a high environmental adaptability. Its distribution range in Ecuador is between the 0 and the 3000 m.a.s.l. (Coloma et al., 2014).

New results on the phylogenetic relationships inside the *R. marina* species complex have been published recently, showing that there are two cryptic species present in Ecuador. Thanks to molecular studies, *Rhinella horribilis* was removed from the synonym of *Rhinella marina* by Acevedo, Lampo and Cipriani (2016) determining that they were

different species and revalidated the species *Rhinella horribilis* for the populations of Central America and South America, distributed to the West of the Andes, setting this species' distribution in the coastal region of Ecuador. After this evaluation, *R. marina's distribution includes* the east of the Andes, in the Ecuadorian Amazon region, Brazil, Colombia, Peru and Bolivia. It is an invasive species introduced worldwide, including Hawaii, Fiji, Philippines, Taiwan, Japan, Australia and many other (Acevedo et al., 2016; Frost, 2016; Jolly et al., 2015; Vallinoto et al., 2010). *Rhinella horribilis,* species that was part of the *R. marina,* is now distributed in Texas, Mexico, Central America, Colombia and Ecuadorian coast, at least until more studies are carried out, clarifying the convoluted taxonomic relationships of this species complex (Acevedo et al., 2016; Frost, 2016).

This study sought to investigate the ranges of thermal tolerances of the larval stage of *Gastrotheca riobambae* and *Rhinella marina,* acclimated to different temperatures, in order to obtain their capacity of acclimation and, with this data, to estimate the relative vulnerability of both species towards climate change, through comparison of their tolerance ranges obtained to their microclimate temperatures.

3.2. Material and methods

3.2.1. Study species

One hundred tadpoles for each species were collected from localities at Pichincha province. *Gastrotheca riobambae* was collected from the Metropolitan Park of Quito, at 2960 m a.s.l. (0º 11′ 1.536′′ S; 78º 27′ 51.011′′ O), while *Rhinella marina* was collected in Mindo, at 1206 m a.s.l. (0º 2′ 52.692′′ S; 78º 47′ 16.187′′ O). Fishnets were used directly in the ponds where the tadpoles were found, and then they were transferred to plastic recipients hermetically closed, labeled with their collection locality and date, and finally transported to the Ecophysiology Laboratory, at Pontificia Universidad Católica del Ecuador in Quito, where the experimental part of this study was developed.

Study organisms were kept at room temperature (20 ºC), with photoperiod 12:12 L:D. The tadpoles of *R. marina* were tested in Gosner stages of 26 to 33, and the tadpoles of *G. riobambae* were between 35-38 Gosner stages (Gosner, 1960).

3.2.2. Acclimation treatments

Tadpoles of both species were exposed to different acclimation temperatures. Twenty tadpoles were randomly distributed into the different acclimation temperatures, individualized in plastic cups, with food *ad libitum* and permanent oxygenation. The temperatures used were 14 ºC, 20 ºC, 26 ºC, 32 ºC and 36 ºC. Ten individuals from each treatment were randomly designated for the upper thermal limit test (CTmax) and ten for the lower thermal limit (CTmin). Each individual was only tested once. All individuals were acclimated for 3 days, being tested on the fourth day according to existing protocols of acclimation (Lutterschmidt and Hutchison, 1997; Westmoreland, 1994).

3.2.3. Thermal tolerance

3.2.3.1. Critical Temperature experimentation

Thermal tolerance tests were conducted using a thermal water bath (HUBER D77656 Offenburg) that has an automatic mechanism for gradually raising or decreasing water

temperature at a previously fixed constant rate of 0.25 ºC per minute. Tadpoles were placed in plastic beakers of 100 ml for the experiment.

Tests were conducted using the dynamic method Lutterschmidt and Hutchinson (1997), in which the tadpoles were heated or cooled, according to the test, until they reached their critical temperature. The definition of critical temperature for ectotherms is "the temperature at which have little or no ability to increase their rate of O_2 consumption above their resting rate, making them incapable of much activity" (Hill et al., 2012). In contrast with critical temperature, we have also the pejus temperatures, that means "turning worse", which are "a range of body temperatures in which the animal deteriorates as body temperature is gradually raised (upper pejus range) or lowered (lower pejus range)" (Hill et al., 2012). The endpoint of the experiment corresponded to the temperature at which the organism did not respond to external stimuli, which was in this case, touching the tadpole with a wooden stick every 20 seconds when the individual started showing lack of movement, until no response was seen, giving us the critical temperature at which the animal is ecologically or behaviorally dead, but not physiologically dead according to Brattstrom (1968). The temperature was taken from the water around the tadpole with a rapid response Miller & Webber mercury thermometer. After the experiment the animal was set into a recipient with water at room temperature, where it could recover from the test. The values were only considered valid when the individual survived 24 hours after the test, to avoid using data related to the lethal temperature of the specimen (Brattstrom, 1968; Hutchison, 1961). CTmax and CTmin of each acclimation temperature are presented as the mean value of the valid experiments. All tadpoles were euthanized at the conclusion of the experiments, according to laboratory protocols based on a lethal dose of MS-222 anesthesia (Canadian Council on Animal Care, 2002; Simmons, 2002).

3.2.3.2. Statistical Analysis

A Shapiro-Wilk's test was used to evaluate the normality of the data and a Bartlett's test was used to prove the homogeneity of variances. If the results were not significant, outliers were removed. Both tests were run again. If the results did not change, a log10 transformation of the data was used to adjust and improve normality and homogeneity of variances. These tests were made for fitting the requirements needed to perform an Analysis of Variance (ANOVA) for testing the differences between the mean values of the

thermal tolerances at the distinct acclimation temperatures, and testing differences between both study species. Depending if the data met the requirements needed, an ANOVA or Kruskal-Wallis (non-parametric) tests were used. An ANCOVA was included in the analysis to improve results, having the weight of the tadpole as a covariable, CT as the dependent variable and the acclimation temperatures as the independent variable.

Finally, depending on the results, the post hoc Tukey's HSD test for the parametric tests, or a Dunn test, for the non-parametric were performed, to determine which treatment (Acclimation temperature) showed differences from the others.

3.2.3.3. Acclimation capacity

Thermal range for both species was determined for each acclimation temperature, by the difference between CTmax and CTmin (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Navas et al., 2008). According to Claussen (1977), Gunderson and Stillman (2015), plasticity for both thermal limits can be measured, calculated as the acclimation response ratio (ARR), defined as the change in the CT per degree of change in acclimation temperature. It is a dimensionless variable, in which ARR=1 indicates a positive acclimation. One °C degree shift in thermal limit for each one °C degree shift in acclimation temperature, suggesting complete balance for acclimation temperature, on the contrary, ARR=0 suggests that there was no acclimation capacity.

3.2.4. Micro environmental data

At the collection points, temperature data loggers (HOBO Pendant® Data Logger) were placed inside the collection ponds, allowing us to gather data on micro environmental conditions. The sensors collected data every 15 minutes for a determined period of time. This procedure allows estimating thermal exposure extremes that these species face at their natural habitats.

For *Rhinella marina*, two places were monitored with data loggers, as we found this specie in both: a dry place with small ponds, clearly disturbed by human presence, with shrub vegetation, near the road, with direct sunlight exposure (from March to July, 2016); and in

little ponds next to a river that meant the pond had little flowing water (from December, 2014 to March, 2015).

For *Gastrotheca riobambae*, two places were monitored with data loggers, one in the collection site, the Metropolitan Park, and the other in a pond at the Pontificia Universidad Católica del Ecuador. Only one data logger could be retrieved, containing data from November 2014 to July 2015, located at the University campus in a small pond filled with *Azolla* sp.

3.2.5. Relative vulnerability to climate change

For analyzing the relative vulnerability of the study species, the warming tolerance (WT) was calculated, which is the difference between the Maximum CTmax obtained for the species, and the maximum exposure temperature (Te) at the micro environmental data at each collection site (Deutsch et al., 2008).

3.3. Results

3.3.1. Thermal tolerance

3.3.1.1 Rhinella marina

The mean CTmax at the different acclimation temperatures, and its standard deviation (SD) showed in Table 1 and Figure A.1, demonstrate that they increase in heat resistance as the acclimation temperature increases. For CTmin, a similar pattern was seen but only for the acclimation temperatures of 20 ºC, 26 ºC and 32 ºC, because the acclimation temperature of 14 ºC, described an inverse trend, losing cold resistance (Table 1, Figure A.2).

For the statistical analysis, the results of the parametric test for this specie showed there was a significant difference of the critical thermal limits between acclimation treatments (ANCOVA, $F= 49.0768$, $p<0.001$). Taking in account the covariable of weight, the body mass of the tadpoles of this specie was statistically significant ($p<0.01$), showed in Appendix A. A *post-hoc* Tukey's HSD test was used to compare differences between acclimation treatments. The comparison between acclimation temperatures of 14 ºC and 20 ºC, and between 26 ºC and 20 ºC were not statistically significant (see Appendix D).

Due to the results of the tests for normality and homogeneity of variance (see Table 2), a non-parametric Kruskal-Wallis test was performed for the CTmin of *R. marina* (Kruskal-Wallis, chi-squeared=20.55, DF=3, $p<0.01$). Results showed the significant difference between each thermal limit for the distinct acclimation temperatures (see Appendix B). A Dunn test was performed for the CTmin acclimation treatments, giving the result of statistical significant difference between the treatments of 14 ºC and 20 ºC, 20 ºC and 32 ºC and 26 ºC and 32 ºC (see Appendix E).

The breadth of thermal tolerance for the different acclimation temperatures of this specie is summarized in Table 3. The lower range of tolerance was for the acclimation temperature of 36 ºC (34 ºC), followed by acclimation 14 ºC with 34.5 ºC. The treatment with the larger tolerance range was 20 ºC with 36.9 ºC of thermal breadth.

3.3.1.2. Gastrotheca riobambae

For CTmax, in Figure B.1, and Table 3, we can observe that heat resistance increases with each acclimation temperature. The lower range of tolerance for CTmax is for treatment 14 ºC with 39.6 ºC, and the highest is the treatment 26 ºC with 40.15 ºC. For CTmin, in Figure B.2, we could observe that for the lowest acclimation temperature (14 ºC), again an inverse trend occurs. Same pattern as observed in *R. marina.* The treatment with the higher range is 14 ºC with 6.7 ºC and the lowest range was for treatment 20 ºC with 2.1 ºC of mean CTmin.

The results of the ANCOVA for CTmax (ANCOVA, $F=12.5147$, $p<0.01$) and the Kruskal-Wallis for CTmin showed thermal acclimation for this specie (Kruskal-Wallis, Chi-squared=20.55, DF=3, $p<0.01$), shown in Appendix B and C. The covariable of weight was not statistically significant in the ANCOVA ($p=0.254$). The *post-hoc* Tukey's HSD Test performed for CTmax, showed that the comparison between acclimation temperatures of 14 ºC and 20 ºC, and between 26 ºC and 20 ºC were not statistically significant (see Appendix F). Dunn Test for CTmin showed significant difference in the paired-comparisons of treatments 14 ºC- 20 ºC, 20 ºC- 32 ºC, 14 ºC- 26 ºC, 26 ºC- 32 ºC (see Appendix G).

G. riobambae did not bear the acclimation phase for the treatment of 36 ºC, the individuals died at the second day, leaving us with the other four treatments (14 ºC=37.4 ºC, 20 ºC=39.9 ºC, 26 ºC=40.3 ºC, 32 ºC=38.2 ºC). The breadth of thermal tolerance for the different acclimation temperatures of this specie is in Table 3. The largest thermal breadth is at 26 ºC of acclimation with 40.3 ºC. The lowest thermal breadth is at 14 ºC, with 37.4 ºC.

3.3.2. Acclimation capacity

The Acclimation Response Ratio (ARR), showed in Table 4, indicates that for the upper limit *Rhinella marina* has a ARR_{MAX} higher that *Gastrotheca riobambae*, ($ARR_{MAX}= 0.20$; $ARR_{MAX}= 0.12$, repectively). For the lower thermal limit, *G. riobambae* has more

acclimation response ratio for CTmin than *R. marina* (ARR_{MIN}= 0.46; ARR_{MIN}= 0.28, respectively).

3.3.3. Micro environmental data

From the micro environmental data, at the collection points we gathered different information. For *R. marina,* we had 4 collection points, and the maximum data obtained was 36,95 ºC. For the same point, its minimum was 18.43 ºC. The variation range for these sites was between 14 to 18 ºC approximately, meaning that each site presented a thermal breadth of 14-18 ºC between its maximum and minimum data. The highest mean of the four sites is 23.75 ºC, and the lowest mean is 20.13 ºC.

For *G. riobambae*, the collection site showed a maximum temperature of 17.38 ºC, a minimum temperature of 8.9 ºC, a range between these data of 8 degrees of variation, and a mean of 14 ºC, showing that this micro environmental data have less variation than the ones obtained in Mindo for *R. marina*. See Table 6 for more information of the micro environmental data.

3.3.4. Relative Vulnerability to climate change

In Table 5 we can observe for *R. marina* presents the highest values of CTmax of both species. The CTmax used in calculating vulnerability is the maximum CTmax obtained (CTmax= 44.4 ºC from the acclimation treatment of 32 ºC). *G. riobambae* has a maximum CTmax of 41 ºC at the acclimation treatment of 32 ºC. The Warming Tolerance value (WT=7.5 ºC, for *R. marina*; WT=23.6 ºC, for *G. riobambae*) was obtained using the maximum Te, which is the highest environmental temperature obtained from the micro environmental data of the data loggers (Te=36.9 ºC, for *R. marina*; Te=17.4 ºC, for *G. riobambae*).

3.4. Discussion

The study of thermal physiological limits of amphibians is a starting point for understanding the conditions that species need in their environment, and allows us to have estimations of their vulnerability to the impacts of anthropogenic climate change in our planet (Charmantier et al., 2008; Deutsch et al., 2008; Duarte et al., 2012; Gerick et al., 2014; Pounds et al., 2006; Tejedo et al., 2012).

Several studies suggest that tropical species could be at higher risk, living near or already exposed to their thermal limits, mainly because they live in warmer habitats and also because annual climatic variation is lower in the tropics. Compared to species of higher latitudes, meaning that tropical species naturally bear less seasonal environmental variations than species living in temperate zones (Duarte et al., 2012; Gunderson and Stillman, 2015; Gutiérrez-Pesquera et al., 2016; Janzen, 1967).

Aquatic larval amphibians are an ideal model to analyze thermal adaptations (Gutiérrez-Pesquera et al., 2016), because they are ectotherms that may be forced to bear the temperatures that their environment may present (temporary and ephemeral ponds, often exposed to direct sunlight, presenting a wide range of temperatures) (Duarte et al., 2012; Watson et al., 1995), without having the opportunity for searching more favorable microhabitats, forcing them to adjust their physiological limits, bearing thermal stress and acquiring thermal resistance through thermal selection (Gutiérrez-Pesquera et al., 2016; Tejedo et al., 2012). The tadpoles of the study species *Gastrotheca riobambae* and *Rhinella marina* frequently can be found in this kind of temporary, shallow ponds (Coloma et al., 2014; Duellman, 2015; Duellman and Trueb, 1986; Frost, 2016) making them interesting species for thermal tolerance analysis.

Different studies show that climate change is going to increase mean environmental temperatures. By the year 2100, there is a projection of an increase of 4.3 ± 0.7 ºC of global temperature, and also high probability of extreme thermal events (Gutiérrez-Pesquera et al., 2016; Intergovernmental Panel on Climate Change, 2014; Pacifici et al., 2015). Acclimation, adaptation, dispersal and behavioral plasticity will help the organisms to cope with the adverse impacts of climate change (Deutsch et al., 2008). Studying the species vulnerability to climate change is a way of analyzing which species could present

higher risk and according to different studies, ectotherms with low dispersal ability as amphibians could get more affected (Duarte et al., 2012; Gerick et al., 2014; Gutiérrez-Pesquera et al., 2016; Huey et al., 2012).

In our study, both species showed a positive acclimation response (see Table 4), as shown by the ARR values. *G. riobambae* presented higher ARR_{MIN} (0.46), which means that this specie has more acclimation capacity for the lower thermal limit, in comparison to the ARR_{MIN} (0.28) of *R. marina.* This could be explained due to its natural distribution is in the highlands and interandean valleys of Ecuador (Duellman, 2015; Frenkel et al., 2014). *R. marina* has a higher ARR_{MAX} (0.2) than *G. riobambae* (ARR_{MAX}=0.12), because it is a widely distributed species in Ecuador, Central and South America, thanks to this fact this species is exposed to a wide set of environmental temperatures, making it a successful invasive species in Australia (Coloma et al., 2014; Tingley and Shine, 2011).

R. marina, or the cane toad, is an interesting amphibian that has experienced an extraordinary success. It is one of the world's most widespread invasive species. Since its introduction in 1935 in northeastern Queensland, Australia, cane toads have spread throughout tropical and temperate (southwards) Australia (McCann et al., 2014). It has even shifted its climatic niche from its native well-watered tropical and subtropical region, to the driest continent on earth, mainly by behavioral plasticity. They compensate their lack of morphological of physiological means to reduce desiccation by sheltering themselves in damp sites for days or weeks and only moving between shelter sites when there are favorable rainy conditions (Tingley and Shine, 2011). The ARR_{MIN} of *R. marina* is higher that its ARR_{MAX}, and according to McCann et al. (2014), this species shows a peculiarity of having a rapid acclimation for cold (12h hours minimum), that is one of the characteristics that allows it to invade high-elevation sites in southeastern Australia (Jolly et al., 2015).

Even though all values of ARR were positive, all four are closer to 0 than to 1, showing a low acclimation capacity in thermal tolerance for both species, and the highest value obtained, 0.46 for the ARR_{MIN} of *G. riobambae* fails to pass over 0.5. This means that if the environmental temperature approaches the thermal limits of this species, that plasticity would not be enough for the specie to survive (Gunderson and Stillman, 2015). In comparison with another similar study (Escobar, D., unpublished data, 2016), the ARR

obtained for other Ecuadorian species, *Agalychnis spurrelli* (ARR_{MAX}=0.14) and *Gastrotheca pseustes* (ARR_{MAX}=0.05), our study species have similar ARR values. Having low ARR values follows the predictions for tropical species that live in more stable thermal conditions that temperate species. Tropical lowland species are expected to be more affected by climate change, because they present narrower thermal tolerance breadth, in comparison to temperate species, mainly because they have thermal stability in their natural environment (Gutiérrez-Pesquera et al., 2016; Pacifici et al., 2015). Having low ARR values show that they have low acclimation capacity, because in their natural environment, equatorial or tropical species don't outstay drastic thermal variations like species that bear the changes between spring and winter.

Both species showed a wide thermal range. *G. riobambae* had the widest with 40.3 degrees of thermal range, meaning they have a 40 degrees difference between its critical thermal maximum and minimum (at an acclimation temperature of 26 ºC). A possible explanation for these results could be the natural environment at which both species are exposed. These species have adapted, via natural selection, over many generations, to the dynamic complexity of their thermal natural environments, consistently experiencing variations in temperature, e.g. *R. marina* has a wide range of temperatures in one collection site of 18.52 ºC (see Table 6) (Angilletta, 2009; Navas et al., 2008). Both species can be found in disturbed areas, have their tadpoles in open spaces, shallow ponds directly exposed to sunlight and to wide variations in water temperature (Coloma et al., 2014; Duellman, 2015; Frenkel et al., 2014; Frost, 2016). Tadpoles that live in temporary ponds at the tropical region can bear high exposure temperatures, like *Lepidobatrachus llanensis*, from Paraguay and Argentina that shows a CTmax of 44.7 ºC, being a subtropical species (Duarte et al., 2012) or *Smilisca phaeota* with 44.0 ºC for a tropical species (López Rosero, 2015), and *Rhinella marina* with 44.4 ºC of the higher CTmax obtained in this study.

Rhinella marina from south Texas, USA, through tropical Central America, up to the Bolivian and north Peruvian amazon regions, has the widest distribution range. Now it is divided with the range of *R. horribilis*, but it is still a wide distribution. It is an invasive species, not only in Australia, as mentioned before, but also in Hawaii, several tropical and subtropical islands and localities (e.g. Haiti, Barbados, Aruba), and even Taiwan (Solís et al., 2009). Being an ectotherm, its body temperature is directly correlated to the external environmental temperature (McCann et al., 2014), this is why *R. marina* was expected to

be the specie with the wider thermal tolerance range, but our results showed differently, being *G. riobambae* our species that has the widest tolerance range and also the highest value of ARR (see Table 3 and 4).

The CTmax and CTmin obtained for different acclimation temperatures for both study species are summarized in Table 1. CTmax in both species increases according to the acclimation temperatures, but for CTmin, in the lowest acclimation temperature (14 ºC), there is an inverse trend. This could be explained by the decrease of physiological performance due to low temperatures. At this acclimation temperature, organisms could start to fail; in a way they could be near their critical thermal minimum, reaching to its lower pejus range (Hill et al., 2012; Huey and Stevenson, 1979; Tejedo et al., 2012). The statistical analysis suggested that there is a significant difference between the acclimation temperatures, and that both species do show an acclimation capacity, meaning that there is a difference between the critical temperature limits obtained in the different acclimation temperatures (Gutiérrez-Pesquera et al., 2016; Westmoreland, 1994).

When we compare figures A.1 with B.1, it is evident that, *R. marina* has a higher tolerance to heat, while comparing figures A.2 and B.2, we can observe that the more cold tolerant species is *G. riobambae*. This could be explained through a theory that declares that species of high altitude, thermodynamically behave as temperate species (Brattstrom, 1968), and temperate species usually present more thermal tolerance to cold, while tropical species have affinity to present more thermal tolerance to heat (Bernal and Lynch, 2013; Gutiérrez-Pesquera et al., 2016; Janzen, 1967; Navas et al., 2010).

Calculating the relative vulnerability of the species helps us to assess the possible effects of climate change in these Ecuadorian species (Gunderson and Stillman, 2015; Parmesan, 2006). The difference between CTmax and the maximum exposure temperature obtained of the micro environmental data (Te) allows for understanding of how close these species are right now to their thermal tolerances. Neither of our study species faces temperatures in their microhabitat data that are close to their thermal limits. The species with a narrower warming tolerance is *R. marina*, making this the most vulnerable specie of this study. Even though it has a WT of 7.45, it is not considered a species at risk. Its conservation status is Least Concern according to the UICN, due to its wide distribution, acclimation capacity, adaptability to different habitats (Coloma et al., 2014; Frost, 2016; Solís et al., 2009).For

G. riobambae, the WT is even wider, of 23.6 ºC, far from its thermal limit, making this the least vulnerable specie of this study.

Being the least vulnerable species of the study indicates that *G. riobambae* may not be affected by an increase in their environmental temperature. *G. riobamabe*, it has a Conservation Status of Vulnerable according to the UICN list, mainly because of human population growth, habitat loss, use of pesticides that are a problem for the health of *G. riobambae*'s remaining populations (Frenkel et al., 2014). Global warming could affect this species in an indirect way through chytrid fungus (Lötters et al., 2009). *Batrachochytrium dendrobatidis* and climate change may enhance this pathogen (Cooke et al., 2013; Pounds et al., 2006). According to the study of Manzano (2010) and Narváez (2014) the population present at the same collection point of our study organisms, (Metropolitan Park of Quito) show the presence of the chytrid fungus within 33,9% of the tadpoles infected. The proportion of the infected tadpoles correlates with the temperature of water; there was more infection at higher temperatures.

A final concern is that the micro environmental data may be underestimated having new high records for the global temperature each month of this last year 2016 (Lynch, 2016). The micro environmental data for *G. riobambae* were taken from November of 2014 to September of 2015, but due to high temperatures, no rainfall and no presence of water in the collection site, experimentation with this species could not be finished until January of 2016. This evidences that this species goes through considerable variations of exposure temperatures in their natural environments.

To asses if our study species are going to be affected or benefited by climate change, it is necessary to know other physiological data like the (Gerick et al., 2014). Unpublished data (Pintanel. P., 2017), compared to the mean Te of each microenvironmental data (Te=23.8 ºC, for *R. marina*, and Te= 14.18 ºC for *G. riobambae*), gave an interesting result: for both of our species, climate change may be beneficial, allowing them to be closer to their T_{opt}, and enhancing their fitness (Angilletta, 2009).

3.5. Conclusions

The trends we can observe in our results show us that *R. marina* is more tolerant to heat, with a maximum CTmax of 43.89 ºC, compared to the maximum CTmax of *G. riobambae* of 40.11 ºC.

G. riobambae is more tolerant to cold, presenting the lowest CTmin of 2.13 ºC, compared to the lowest CTmin of *R. marina* of 7.28 ºC.

The thermal tolerance show that *G. riobambae* has the wider range (40.3 degrees for treatment 26 ºC) in all of the acclimation temperatures, compared to the wider range of *R. marina* (36.9 degrees for treatment 20 ºC), even though this species is not good acclimating at higher temperatures, the treatment of acclimation at 36 ºC failed, but it can bear a great range of different temperatures.

The narrowest thermal tolerance range was of *R. marina* with 34 degrees for the treatment of 36 ºC of acclimation.

Gastrotheca riobambae had the highest acclimation capacity for its lower thermal limit (ARR_{MIN}=0.46), being the species that could better acclimatize for the cold thermal limit, compared to the ARR_{MIN}=0.28 of *Rhinella marina*.

For the high thermal limit, *R. marina* had a better acclimation capacity (ARR_{MAX}=0.2) than *G. riobambae* (ARR_{MIN}=0.12).

Micro environmental data showed that the most variable localities are the ones where the data for the cane toad was collected. It also indicates that *G. riobambae* has a narrow Te range with only 8 degrees between the highest and lowest record, because of this; we suggest that more environmental data, probably in other localities, should be gathered for evaluating this species. The Te ranges showed that more environmental variability was seen in Mindo. The highest mean of Te was for Mindo with 23.8 ºC, while the mean for Quito was 14 ºC.

For our data, the widest WT is for *G. riobambae* (WT=23.6) and the smallest is for *R. marina* (WT=7.5), indicating that *R. marina* is the most vulnerable species of this study, showing the lowest value for relative vulnerability.

Given the WT values obtained, neither of our study species are living near their thermal tolerances and probably won't be affected by changes in environment, at least not in a direct way. These species do not show immediate vulnerability to climate change in collection sites where this study was made. If the T_{opt} data are confirmed for these two species they could even be benefited if their exposure temperature gets closer to their T_{opt}.

4. APPENDICES

Appendix A. Results of Analysis of Covariance (ANCOVA) for CTmax ~ Acclimation Temperatures in *Rhinella marina.*

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	
Acclimation Temperatures	3	38.177	12.7255	49.07680	2.911E-12	***
Weight	1	1.528	1.5279	5.8924	0.020826	*
Acclimation Temperature - Weight	3	3.539	1.1796	4.5491	0.008938	**
Residuals	33	8.557	0.2593			

Significance 0 '*' 0,001 '**' 0,01 '*' 0,05 '.' 0,1 ' ' 1**

Appendix B. Results of Kruskal–Wallis (Non-parametric test) of CTmin ~ Acclimation Temperatures in *Gastrotheca riobambae* and *Rhinella marina.*

Kruskal- Wallis

Specie	CT	Chi-squeared	Degrees of freedom	P-value
G.riobambe	min	20.55	3	0.0001305
R.marina	min	24.346	3	2.12e-05

Appendix C. Results of Analysis of Covariance (ANCOVA) for CTmax ~ Acclimation Temperatures in *Gastrotheca riobambae*

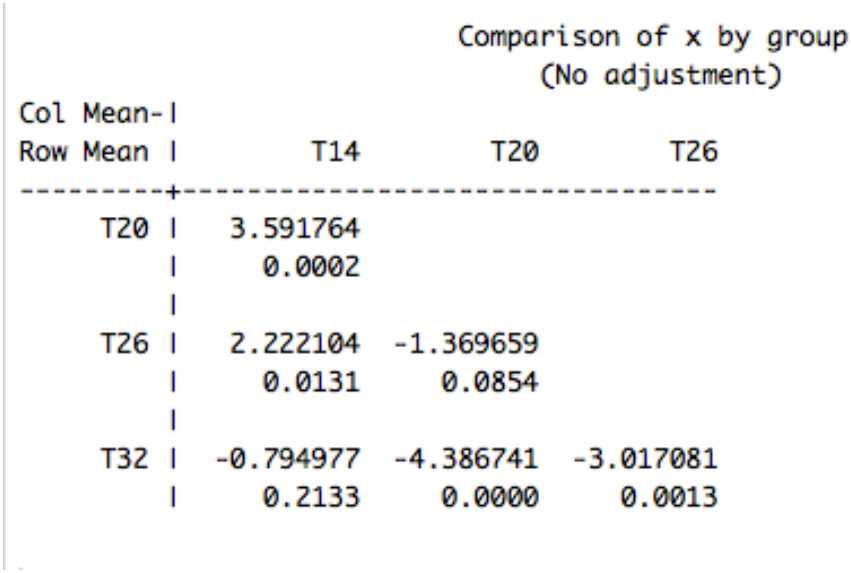
	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Acclimation Temperatures	3	5.6452	1.88173	12.5147	1.57E-05	***
Weight	1	0.1802	0.18016	1.1982	0.2821	
Acclimation Temperature - Weight	3	0.6432	0.21439	1.4258	0.254	
Residuals	31	4.6612	0.15036			

Significance 0 '*' 0,001 '**' 0,01 '*' 0,05 '.' 0,1 ' ' 1**

Appendix D. *Post hoc* Tukey's HSD Test of CTmax ~ Acclimation Temperatures in *Rhinella marina*

Tukey multiple comparisons of means				
95% family-wise confidence level				
Treatments	**diff**	**lwr**	**upr**	**p adj**
20 ºC-14 ºC	0.8363636	0.1232330	1.5494940	0.0161089
26 ºC-14 ºC	1.3100000	0.5800875	2.0399120	0.0001365
32 ºC-14 ºC	2.6900000	1.9600875	3.4199120	0.0000000
26 ºC-20 ºC	0.4736364	-0.2394943	1.1867670	0.2958648
32 ºC-20 ºC	1.8536364	1.1405057	2.5667670	0.0000002
32 ºC-26 ºC	1.3800000	0.6500875	2.1099120	0.0000620

Appendix E. Dunn Test of Multiple Comparisons Using Rank Sums of CTmin ~ Acclimation Temperatures in *Rhinella marina.*



Comparison of x by group
(No adjustment)

Col Mean- Row Mean	T14	T20	T26
T20	3.591764 0.0002		
T26	2.222104 0.0131	-1.369659 0.0854	
T32	-0.794977 0.2133	-4.386741 0.0000	-3.017081 0.0013

Appendix F. *Post hoc* Tukey'S HSD Test of CTmax ~ Acclimation Temperatures in *Gastrotheca riobambe*

Tukey multiple comparisons of means **95% family-wise confidence level**				
Treatments	**diff**	**lwr**	**upr**	**p adj**
20 ºC-14 ºC	0.1600000	-0.3174380	0.6374380	0.8028900
26 ºC-14 ºC	0.6100000	0.1325620	1.0874380	0.0077964
32 ºC-14 ºC	0.9822222	0.4917013	1.4727431	0.0000274
26 ºC-20 ºC	0.4500000	-0.0274380	0.9274380	0.0706878
32 ºC-20 ºC	0.8222222	0.3317013	1.3127431	0.0003786
32 ºC-26 ºC	0.3722222	-0.1182987	0.8627431	0.1908551

Appendix G. Dunn Test of Multiple Comparisons Using Rank Sums of CTmin ~ Acclimation Temperatures in *Gastrotheca riobambae.*

Comparison of x by group
(No adjustment)

Col Mean- Row Mean	T14	T20	T26
T20	3.827974 0.0001		
T26	2.737001 0.0031	-1.090972 0.1376	
T32	0.363657 0.3581	-3.464317 0.0003	-2.373344 0.0088

5. FIGURES

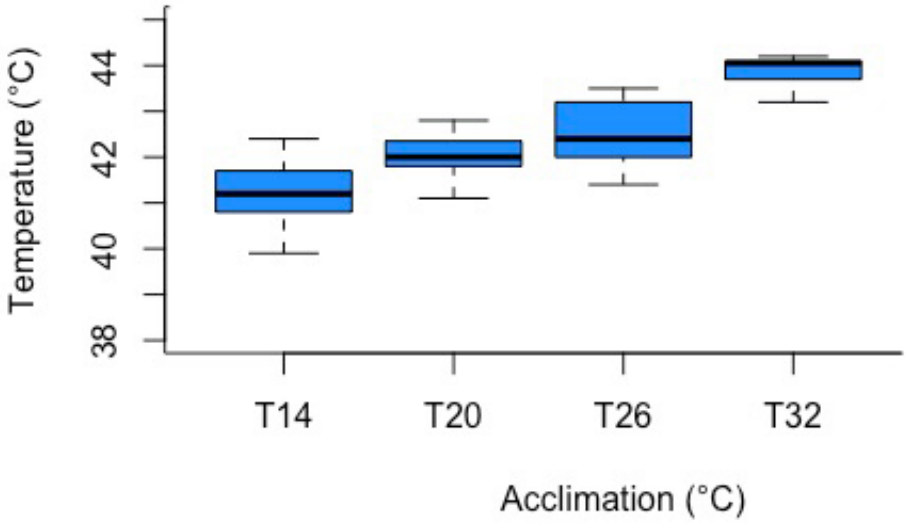


Figure A.1. Variation of Critical Thermal maximum (CTmax) in *Rhinella marina* at different treatmens of Acclimation temperatures (14 ºC, 20 ºC, 26 ºC, 32 ºC).

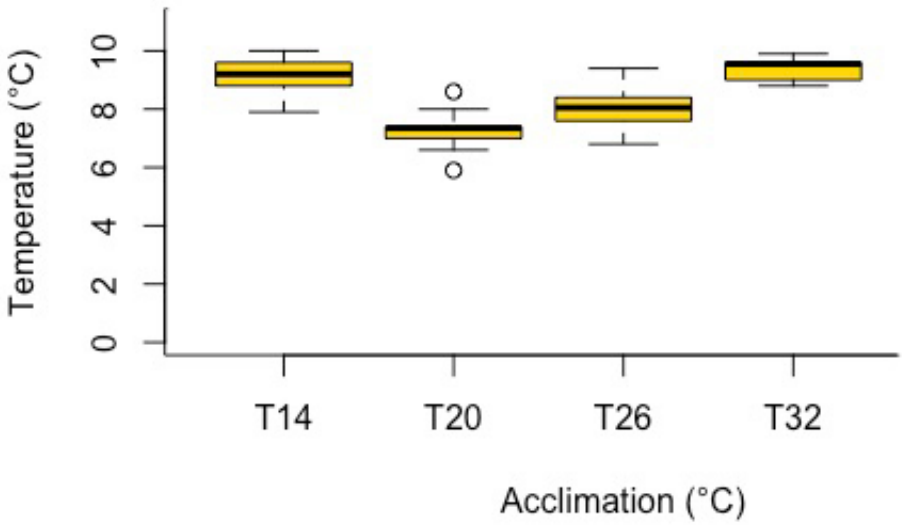


Figure A.2. Variation of Critical Thermal minimum (CTmin) in *Rhinella marina* at different treatments of Acclimation temperatures (14 ºC, 20 ºC, 26 ºC, 32 ºC).

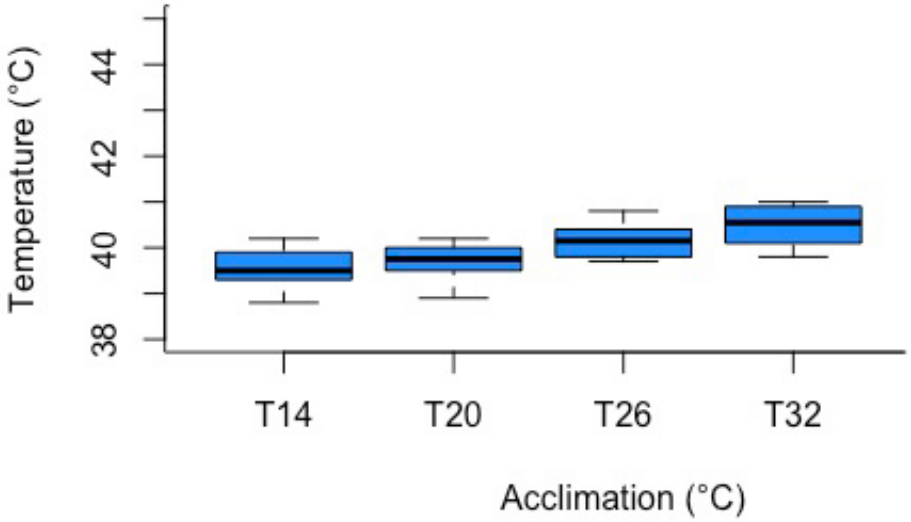


Figure B.1. Variation of Critical Thermal maximum (CTmax) in *Gastrotheca riobambae* at different treatments of Acclimation temperatures (14 ºC, 20 ºC, 26 ºC, 32 ºC).

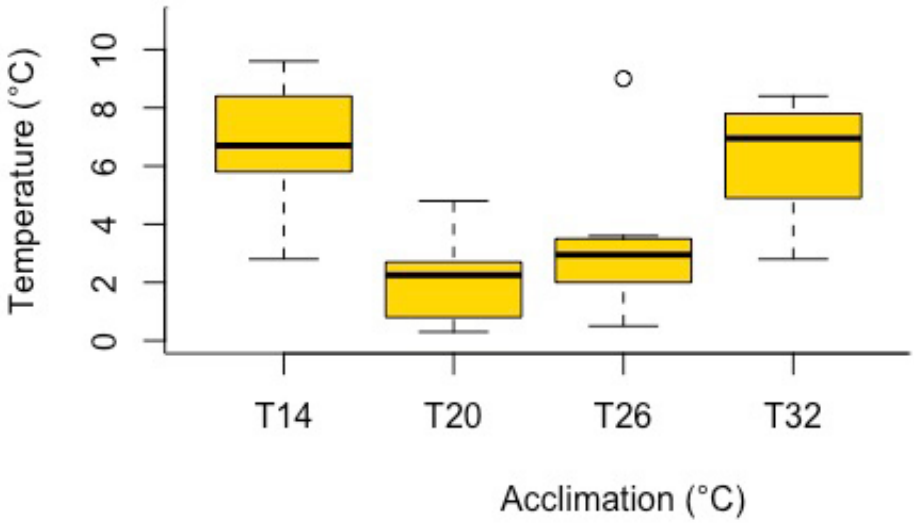


Figure B.2. Variation of Critical Thermal minimum (CTmin) in *Gastrotheca riobambae* at different treatments of Acclimation temperatures (14 ºC, 20 ºC, 26 ºC, 32 ºC).

Figure C.1. Carolina Yandún-Vela, for the section Vitae.

Figure C.2. Andrés Merino-Viteri, for the section Vitae.

6. TABLES

Table 1. Thermal tolerances: Critical Thermal maximum and Critical Thermal minimum, mean and standard deviation of each acclimation temperature treatment of species *Gastrotheca riobambae* and *Rhinella marina*.

Species	Acclimation Temperature	Ctmax (ºC±SD)	N	CTmin (ºC±SD)	N
Rhinella marina	14 ºC	41.2±0.709	10	9.1±0.71	10
	20 ºC	42.03±0.52	10	7.28±0.52	10
	26 ºC	42.51±0.77	10	8.07±0.77	10
	32 ºC	43.89±0.33	10	9.45±0.33	10
	36 ºC	43.44±1.20	10	11.15±1.20	10
Gastrotheca riobambae	14 ºC	39.54±0.42	10	6.7±2.05	10
	20 ºC	39.7±0.37	10	2.13±1.45	10
	26 ºC	40.15±0.37	10	3.14±2.31	10
	32 ºC	40.11±1.36	9*	6.23±1.96	10

*Only outlier removed for final statistical tests due to methodological error.

Table 2. Results of statistical tests, normality test (Shapiro-Wilk's) and homogeneity of variances (Bartlett's) with outliers, without outliers and log10 transformation of data if needed for *Gastrotheca riobambae* and *Rhinella marina.* (Not significant highlighted).

		Original data	**Without Outliers**	**Log10 Transformation**
Bartlett's				
Species	**Parameter**	**P-value**	**P-value**	
G.riobambae	CTmax	**1.74e-02**	0.9738	
G.riobambae	CTmin	0.6056	0.4033	
R. marina	CTmax	0.09997	0.09997	
R. marina	CTmin	0.1778	0.1648	
Shapiro-Wilk's				
		p-value	**p-value**	**p-value**
G.riobambae	CTmax	**2.04E-02**	0.7442	
G.riobambae	CTmin	**0.03684**	**0.02696**	**0.01012**
R. marina	CTmax	0.1524	0.1524	
R. marina	CTmin	**0.04984**	**0.029**	**0.002653**

Table 3. Thermal tolerance ranges for the different acclimation temperatures for both species, *Gastrotheca riobambae* and *Rhinella marina*.

Species	**Acclimation Temperature**				
	14 ºC	20 ºC	26 ºC	32 ºC	36 ºC
Gastrotheca riobambae	37.4	39.9	40.3	38.2	-
Rhinella marina	34.5	36.9	36.7	35.4	34

Table 4. Acclimation response ratio (ARR= ΔCTmax or ΔCTmin/ Δ acclimation temperature) in *Gastrotheca riobambae* and *Rhinella marina.*

Species	MeanAcclim. Temp.	Mean Ctmax	ARR_{MAX}	Mean CTmin	ARR_{MIN}
Gastrotheca riobambae	18	2.2	0.12	8.3	0.46
Rhinella marina	22	4.5	0.2	6.2	0.28

Table 5. Warming Tolerance (Maximum CTmax – Maximum Te) for *Gastrotheca riobambae* and *Rhinella marina.*

Species	Maximum CTmax	Maximum Te	WT
Rhinella marina	44.4	36.9	7.5
Gastrotheca riobambae	41	17.4	23.6

Table 6. Summary of Micro environmental data obtained from data loggers at both collection points.

Locality	Maximum (ºC)	Minimum (ºC)	Mean (ºC)	Range (Max-min)	Start Date	End Date
Mindo (Dry)	36.95	18.43	23.75	18.52	10/3/16	26/4/16
Mindo (Dry)	32.39	18.14	23.8	14.25	20/4/16	20/7/16
Mindo (River)	33.85	18.62	22.25	15.23	10/3/16	20/4/16
Mindo (River)	33.95	18.14	20.13	15.81	14/12/14	7/3/15
PUCE_pond	17.38	8.9	14.18	8.48	20/11/14	26/9/15

Informed Consent

All the animal procedures were in accordance with international guidelines. The work described has been carried out in accordance with the *EU Directive 2010/63/EU for animal experiments: http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm.*
All permits needed according to the Ministerio del Ambiente de Ecuador were obtained for collecting, transportation and maintenance of the living organisms. All tadpoles were euthanized at the conclusion of the experiments, according to laboratory protocols based on a lethal dose of MS-222 anesthesia (Canadian Council on Animal Care, 2002; Simmons, 2002), protocols used at the QCAZ Museum of the Pontificia Universidad Católica del Ecuador.

Declaration of Interest

We hereby declare that authors of this publication have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence our work.

Submission declaration and verification

This article has not been published previously (except in the form of an academic thesis of the Pontificia Universidad Católica del Ecuador). It is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly approved by the responsible authorities of the institution where it was developed. If it is accepted by the Journal of Thermal Biology, it will not be published elsewhere in the same form, in English or any other language, including electronically without the written consent of the copyright-holder. To verify originality, this article was checked by an online originality detection service.

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Vitae

Carolina Yandún-Vela is a biology student at the Pontificia Universidad Católica del Ecuador, finishing her thesis project to obtain a bachelor's degree in biological sciences. Born in 1992, at the city of Quito, Ecuador, started her University studies in 2010. Working member of the QCAZ Museum of the PUCE since 2011, at the Herpetology division. My work with amphibians started as a scholarship trainee in the Balsa de los Sapos Conservation Initiative, where I learned more about anurans and wanted to learn more about their ecology. Inspired by amphibians since 2005, thanks to the Sapari exhibition at the PUCE.

Andrés Merino-Viteri is a biologist graduated from the PUCE, born in Quito, Ecuador that has dedicated his life to the study of the ecology of amphibians. He is a member of the Herpetology Laboratory of the QCAZ Museum and current Director of the Balsa de los Sapos Conservation Initiative. He studied at the James Cook University in Australia for his Doctoral degree, and currently he is working in association with the Biological Investigation Estation of Doñana in Spain, in the Ecophysiology Laboratory to obtain viable ecophysiological data that will help to asses the impacts of climate change in amphibian species.

Highlights

- *Rhinella marina* and G*astrotheca riobambae* showed positive acclimation to different temperature treatments.
- Neither of the two species have relative vulnerability to climate change.
- *G. riobambae* has the wider thermal breadth of both species.
- *R. marina* tolerates better the heat, while *G. riobambae* is better adapted to cold

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